REGULAR ARTICLE

QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte "*Zea nicaraguensis*" cross

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Abstract Using a 141 F_2 population generated from maize inbred B64 × teosinte *Zea nicaraguensis* cross, quantitative trait loci (QTLs) controlling aerenchyma formation in roots under non-flooding drained soil conditions were identified. Seedlings of *Z. nicaraguensis* formed clear aerenchyma in the cortex of adventitious roots in non-flooding conditions, whereas the maize inbred line B64 did not. In the F_2 population, the capacity to develop aerenchyma exhibited wide and continuous variation, suggesting the trait was controlled by multiple genes. A linkage map was developed using 85 SSR markers, covering 1,224 cM across all ten chromosomes. Composite interval mapping analysis revealed that four QTLs for aerenchyma formation under non-flooding conditions were located to two regions of chromosome 1 (identified as *Qaer1.02-3* and *Qaer1.07*), chromosome 5 (*Qaer5.09*) and chromosome 8 (*Qaer8.06-7*), and these explained 46.5% of the total phenotypic variance. The multiple interval mapping approach identified additional QTLs on chromosomes 1 (*Qaer1.01*) and 5 (*Qaer5.01*). Using these results, it may be possible to use SSR markers linked to aerenchyma formation in a marker assisted selection approach to introduce aerenchyma formation in drained soil conditions into maize for the eventual development of flooding tolerant maize hybrids.

Keywords Aerenchyma · Flooding · Maize · Quantitative trait locus · Teosinte

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Introduction

Flooded or waterlogged soils are distributed worldwide in crop production areas and can greatly reduce yields (Boyer 1982). In Japan, in order to maximize land productivity, forage crops such as maize and sorghum are required to be grown in non-cultivated upland rice paddy fields (approximately 1×10^6 ha in Japan). The availability of such rice paddy fields are approximately ten times greater than the land area

that is now specifically sown to maize (9×10^4 ha, Statistic Department, Minister's Secretariat, Ministry of Agriculture, Forestry and Fisheries 2005). The cultivation of maize and sorghum are primary forage resources for the dairy industry in Japan. During the rainy season, soil flooding in poorly drained upland paddies is a major source of environmental stress for these forage crops. As a consequence, to increase maize production in these environments, we are attempting to develop flooding-tolerant elite maize lines.

To achieve this goal, we have focused on soil flooding tolerance at the seedling stage since this is the critical point when a field's production potential is most affected. Breeding for soil flooding tolerant maize lines is difficult since multiple factors related to flooding reduce the repeatability of an experiment even at the seedling stage (Mano et al. 2002). In order to enhance the repeatability of the flooding trials, we have divided soil flooding tolerance at the seedling stage into three components: (1) the plant's capacity to form aerenchyma channels in roots (Arikado and Adachi 1955; Armstrong et al. 1991; Burdick 1989; Laan et al. 1989; McDonald et al. 2001); (2) the plant's capacity to grow adventitious roots at the soil surface during flooding (Bird 2000; Lizaso et al. 2001; Visser et al. 1996); and (3) the plant's tolerance to soil flooding at soil reducing or low redox potential (Eh) conditions (Mano et al. 2006a; Pezeshki 2001; Yamasaki 1952); at these conditions, phytotoxins (e.g., Fe^{2+}, H_2S) were induced (Ponnamperuma 1984). Regarding the plant's capacity to form adventitious roots at the soil surface, we have previously identified quantitative trait loci (QTLs) controlling the trait in the genus *Zea* (Mano et al. 2005a, c) and a marker-assisted backcrossing program is now in progress (Y. Mano, unpublished). The importance of both adventitious roots at the soil surface and aerenchyma formation in roots has been reported to be an adaptive plant response to soil flooding. In barley, superior flooding tolerant lines, selected from over 4,000 accessions in soil flooded conditions, were observed to develop and exhibit abundant adventitious roots with aerenchyma (Stanca et al. 2003; Takeda 1989). In addition to the capacity to form adventitious roots at the soil surface, it may be possible to develop superior maize lines tolerant of soil flooding by introgression of the enhanced aerenchyma formation trait.

Teosinte is considered to be the closest, wild relative of maize (*Zea mays* L. ssp. *mays*, Linn.). According to Iltis and Doebley (1980) and Iltis and Benz (2000), the taxonomy of teosinte includes three subspecies of *Zea mays*; (1) *Zea mays* L. ssp. *mexicana* (Schrad.) Iltis, (2) *Z. mays* ssp. *parviglumis* Iltis & Doebley, and (3) *Z. mays* ssp. *huehuetenangensis* (Iltis & Doebley) Doebley and four separate species; (1) *Z. luxurians* (Durieu & Asch.) Bird, (2) *Z. diploperennis* Iltis, Doebley & Guzman, (3) *Z. perennis* (Hitchc.) Reeves & Mangelsdorf, and (4) *Z. nicaraguensis* Iltis & Benz. With the exception of tetraploid *Z. perennis*, the teosintes possess the same diploid chromosome number as maize ($2n = 2x = 20$) and their chromosomes are known to generally pair and recombine with the chromosomes of maize (Molina and Naranjo 1987; Ting 1958). Historically, maize × teosinte hybridizations have provided germplasm for heat and drought tolerance (Reeves 1950), aluminum tolerance (Barcelo et al. 1993) and grain yield production (Cohen and Galinat 1984). Since *Z. nicaraguensis* grows in an environment where flooding is frequent, Iltis and Benz (2000) have postulated that teosinte may be a potentially valuable source of germplasm for the development of maize with soil flooding tolerance.

In an earlier study, using well-aerated, drained conditions, *Z. luxurians* was reported to develop well-formed aerenchyma in adult plants (Ray et al. 1999). Recently, we investigated maize and teosinte seedlings in greater detail with regard to the formation of aerenchyma in several parts of the adventitious roots, and discovered that both teosinte and some flooding-sensitive maize accessions form aerenchyma during soil flooding (Mano et al. 2006b; Y. Mano, unpublished). Flooding-induced aerenchyma formation in maize has been reported in earlier studies (e.g., Drew et al. 1979; Konings 1982). Notably in non-flooded greenhouse condition, *Z. nicaraguensis* and *Z. luxurians* begin to form aerenchyma in adventitious roots 2 weeks after sowing and exhibit an extremely high capacity to form aerenchyma at the seedling stage when compared to maize accessions (Mano et al. 2006b). Flooding tolerant rice (Colmer 2003; Jackson et al. 1985) and some wetland plants (Schussler and Longstreth 1996; Smirnoff and Crawford 1983) have also been observed to form aerenchyma in well-aerated hydroponic solution or drained sand culture. In addition, many wetland species form aerenchyma in

drained soil conditions (Justin and Armstrong 1987). As a consequence, this unique character may be relevant to enhance soil flooding tolerance since a plant that possesses aerenchyma channels in non-flooding drained soil conditions may be able to adapt more rapidly to soil flooding conditions when these occur.

Other than the presumption that only dominant gene(s) are associated with aerenchyma formation in teosinte (Ray et al. 1999), little is known regarding the genetics of the trait in the genus *Zea*. In this study, we report on the identification of QTLs controlling aerenchyma formation at non-flooded drained conditions using a segregating F_2 population derived from a cross between maize and teosinte (B64 × *Z. nicaraguensis*). The identified markers that are linked to aerenchyma formation in drained soil conditions may be useful in the development of soil flooding-tolerant elite maize lines through molecular marker assisted selection.

Materials and methods

Plant materials

Maize inbred line B64 (Accession No. 00094105) was obtained from the Gene Bank, National Institute of Agrobiological Sciences, Tsukuba, Japan, and the teosinte, *Z. nicaraguensis* (CIMMYT 13451), was provided by the International Maize and Wheat Improvement Center (CIMMYT), Mexico. Seedlings of B64 did not form aerenchyma in any portion of the adventitious roots in non-flooding drained soil conditions, while that of *Z. nicaraguensis* formed clear aerenchyma, corresponding to an approximate percentage of aerenchyma in the cortex of 20% when in drained soil (Mano et al. 2006b). Prior to genetic analyses, *Z. nicaraguensis* was self-pollinated in an isolated greenhouse twice in winter season and a stable aerenchyma-forming S2 generation was developed. A single F_1 plant derived from the cross between B64 × *Z. nicaraguensis* was grown in isolation and self-pollinated in the greenhouse during winter. An F_2 population was used to identify QTLs controlling aerenchyma formation in non-flooding drained soil conditions.

In this study, an F_2 mapping population was evaluated for the following reasons: QTL analyses using an F_2 mapping population of the cross between

maize × teosinte have previously been reported (Doebley and Stec 1991, 1993; Bomblies and Doebley 2006), however, the development of F_3 progeny or recombinant inbred lines has not yet been reported due to difficulty of self-pollination in F_2 plants of maize × teosinte cross (J. Doebley, personal communication). For example, self-pollination can be difficult due to teosinte's photoperiodic response. Often F_2 plants do not form tassels during the summer season in Texas or Oklahoma, USA, locations where a maximum day length during the growing season is 14 h (Rogers 1950; B. Kindiger, unpublished). This phenomenon also occurs in the temperate zone of Japan (Y. Mano, unpublished). When the F_2 plants of maize × teosinte were grown in short-day length conditions, these formed tassels and many female spikes, which segregate phenotypically among the F_2 individuals (Doebley and Stec 1991). To avoid out crossing, covering the female spikes is necessary but can be extremely difficult to perform due to the morphology of some F_2 individuals.

In total, for the analysis, 262 F_2 plants were grown in a greenhouse maintained at a temperature of 30°C day/25°C night with natural light at 13–14 h day length, of which 186 grew well and were used for this study. The remaining 76 plants were not used in the experiment due to the occurrence of pale-green chlorophyll variation and subsequently caused necrosis at the early seedling stage (62 plants) or poorly sustained growth (14 plants).

DNA isolation

Approximately 1–4 μg of DNA was isolated from 50 mg of fresh leaf tissue from the parents, F_1 plants and F_2 plants by the method described by Komatsuda et al. (1998).

Root anatomy

Under non-flooding conditions (i.e., in drained soil), aerenchyma channels were observed in the adventitious (shoot-born crown) roots of six-leaf stage seedlings (∼4 weeks old). The seedlings were grown in 11 cm diameter, 30 cm deep pots filled with granular soil (1.2 g N, 5.8 g P, 1.8 g K in each pot) in a greenhouse.

Fresh-root cross sections 80–100 μm thick were made every 5 cm from the root tip using a microtome (MTH-1, Nippon Medical & Chemical Instruments

Co. Ltd., Osaka, Japan). Since the degree of aerenchyma formation in *Z. nicaraguensis* at non-flooding conditions is greater in roots emerging at the second nodes (Mano et al. 2006b), two adventitious roots at the second node were evaluated for the presence of aerenchyma. The amount of aerenchyma in the root cortex was visually scored: 0 (no aerenchyma), 0.5 (partial formation), 1 (radial formation), 2 (radial formation extended toward epidermis) and 3 (well-formed aerenchyma). The averaged scores at 10 and 15 cm from the root tips in two roots (four portions in total) were used for the parents (number of plants = 6 for each), the F_1 plants ($n = 10$) and each F_2 individual for QTL analysis. These locations were used since a higher degree of aerenchyma was observed at these distances. Adventitious roots that were 25–35 cm long were evaluated, as these readily form aerenchyma in drained soils, whereas shorter roots do not (Y. Mano, unpublished). By excluding 45 F_2 plants that exhibited inadequate root length (shorter than 25 cm or longer than 35 cm), a total of 141 F_2 plants out of 186 were evaluated for their capacity to develop aerenchyma formation.

SSR analysis

Based on the SSR list available at the MaizeGDB (http://www.maizegdb.org/ssr.php), a total of 259 SSR primer pairs were surveyed for their quality of PCR amplification and the degree of polymorphism. From these, 85 useful SSR primer pairs were selected to construct a linkage map for the B64 × *Z. nicaraguensis* F_2 population. The SSR analysis was performed as described by Mano et al. (2005b).

Map construction

Following exclusion of the 45 plants that did not exhibit adequate root length, linkage analysis of the remaining 141 individuals in the F_2 population was performed using an F_2 model of MAPMAKER/EXP 3.0 (Lander et al. 1987). Markers were grouped according to a two-point analysis at LOD > 3.0 with a recombination fraction of 0.3. A framework map was constructed according to the "three-point" and "order" command first at the LOD > 3.0 and then at LOD > 2.0. The "ripple" command was used to verify the marker order in the linkage group. Haldane's mapping function was used to calculate map distances.

QTL analysis

Composite interval mapping (CIM) was applied to map the QTLs controlling aerenchyma formation in the F_2 population using the software package Windows QTL Cartographer Version 2.5 (Wang et al. 2006). CIM was run with 2 cM walk speed applying the default parameters (model 6; 5 for control markers, 10 cM for window size and forward regression method) in the program. The experiment-wise significance threshold level was defined as the 50th highest LOD value of 4.1 by running 1,000 permutations, corresponding to an experiment-wise Type-I error rate of 0.05.

Zeng et al. (1999) suggested a limitation to CIM, in that the simple and systematic procedure to map multiple QTL can be affected by an uneven marker distribution (marker-rich/-poor region) in the genome. As an alternative approach to possible CIM limitations, it has been suggested that the multiple interval mapping (MIM) approach could be used for the discovery of additional QTL (Kao et al. 1999). As a consequence, we utilized the MIM approach using the Windows QTL Cartographer. In the MIM analysis, model selection began with an initial genetic model suggested by the CIM results and continued in the search for additional QTL through several cycles using a forward search method. The MIM was performed with 1 cM walk speed applying the penalty function of $c(n) = \ln(n)$ proposed by Schwarz (1978). Kao et al. (1999) described a stepwise procedure to search for additional QTL and an estimation of broad sense heritability ($h_b^2 = V_G/V_P = r^2$) in the MIM model.

Results

Aerenchyma formation

The degree of aerenchyma formation in adventitious roots of plants at the six-leaf seedling stage of the parents, F_1 plants and F_2 population was scored in non-flooding drained soil conditions. When the capacity to form aerenchyma was subdivided by its position in the developing roots of *Z. nicaraguensis*, the F_1 plants and the F_2 population formed more aerenchyma at 10 and 15 cm from the tips of the second node roots than at other distances. Whereas, B64 did not form aerenchyma in any portion of its roots
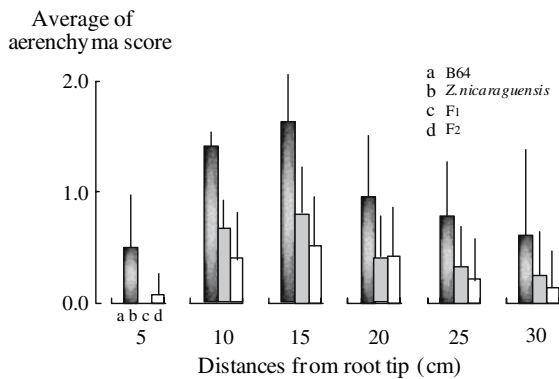
Average of
aerenchyma score

Fig. 1 Capacity of the second-node adventitious roots to form aerenchyma at various distances in non-flooding drained soil conditions in B64 (number of plants = 6), *Z. nicaraguensis* ($n = 6$), their F_1 hybrid ($n = 10$), and F_2 population ($n = 141$). The values are the mean ± standard deviations. Absence of a *bar* indicates no aerenchyma formation (score 0). Of 141 F_2 plants, 116 were evaluated for the capacity to form aerenchyma at every 5 cm from the root tip, whereas the remaining 25 were evaluated only at 10 and 15 cm from the tip. Average lengths of roots evaluated for aerenchyma formation were 28 ± 2 cm (average ± standard deviation) for B64, 30 ± 3 cm for *Z. nicaraguensis*, 29 ± 3 cm for their F_1 plants and 29 ± 3 cm for the F_2 population

(Fig. 1). As a consequence, the average of scores at 10 and 15 cm from the root tips were used in the QTL analysis. Using this criteria, the capacity for aerenchyma formation was 0.0 (mean) for B64, 1.5 ± 0.2 (mean ± standard deviation) for *Z. nicaraguensis* and 0.7 ± 0.2 for the F_1 plants (Fig. 2). The capacity for aerenchyma formation in the F_2 population indicated a continuous distribution with a tendency to reside in the lower end (average 0.5 ± 0.4, Fig. 3). A score of 3 (well-formed aerenchyma) found in *Z. nicaraguensis* during flooded conditions (Mano et al. 2006b) was not identified in any portion of the roots in plants exposed to non-flooding drained soil conditions.

Map construction and segregation distortion

An SSR-based map was constructed using 85 markers, covering 1,224 cM at an average interval of 17.2 cM/locus for the ten chromosomes (Fig. 4). Of these, 5 (5.9%) are dominant markers. By comparing published maize SSR maps (e.g., Sharopova et al. 2002), the marker order is in good agreement with previous maps and the coverage of our map is satisfactory for QTL analysis. On the long arm of chromosome 4, no recombination was found at the region of bin 4.07 (bnlg1784)—bin 4.10 (bnlg1917).
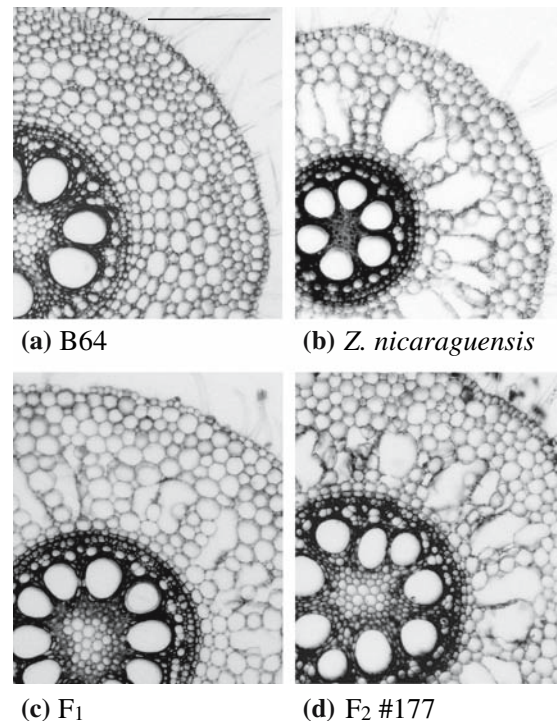

(a) B64　　　　　　　　**(b)** *Z. nicaraguensis*

(c) F_1　　　　　　　　**(d)** F_2 #177

Fig. 2 Cross sections of adventitious roots at 10–15 cm from the root tip that emerged from the second node in six-leaf stage seedlings in non-flooding drained soil conditions. The lack of aerenchyma is shown in (**a**) B64 (score 0) and aerenchyma in (**b**) *Z. nicaraguensis* (score 2), in (**c**) the F_1 plant (score 1) and in (**d**) aerenchyma-forming F_2 plant #177 (score 2). *Bar* = 0.25 mm for all

Segregation ratios at each marker were tested for goodness of fit to the expected 1:2:1 or 3:1 proportions using the chi-square method. For the 85 mapped SSR markers, 8 (9.4%) showed distorted segregation ($P < 0.01$). A total of four regions were indicated to be associated by segregation distortion to: chromosome 1 (the most extreme segregating region being at bin 1.07), chromosome 5 (5.04), chromosome 7 (7.04), and chromosome 8 (8.02) (Fig. 4). Distorted segregation regions on chromosomes 1 and 7 were associated with the lower frequency genotype of *Z. nicaraguensis*, whereas those on chromosome 5 were associated with the lower frequency of genotype of B64. The region on chromosome 8 favored a heterozygous genotype (Table 1).

The regions on chromosomes 1 and 8 were located within the known segregation distortion regions of SDR1.2 and SDR8.1, respectively (Lu et al. 2002). The segregation distortion regions found in this study did not correspond to the gametophytic factor gene,
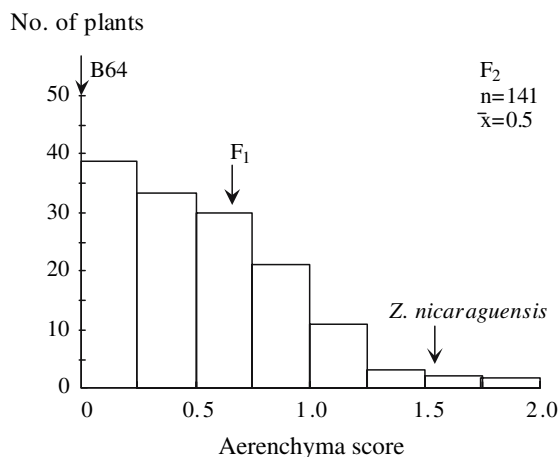
No. of plants

Fig. 3 Frequency distribution for aerenchyma formation of the B64 × *Z. nicaraguensis* F$_2$ population in non-flooding drained soil conditions. An average score of two adventitious roots at 10 and 15 cm from the root tip (four portions in total) was used as the value for each F$_2$ individual. Only adventitious roots exhibiting a length of 25- to 35 cm-long were evaluated

Ga1, reported to be in chromosome 4 (bin 4.01-02) in several teosintes (Doebley and Stec 1993; Mano et al. 2005b; Westerbergh and Doebley 2002). The strongest segregation distortion was found on chromosome 7 ($\chi^2 = 43.9$, $P < 0.001$) and may be caused by the elimination of the *Z. nicaraguensis* necrosis allele that may be associated to the removal of 62 chlorophyll-variant necrotic plants from the study. When the genotype of the most distorted marker "dupssr13" located on chromosome 7 was tested in 24 of the 62 necrotic F$_2$ plants that were not used for map construction and from which DNA was obtained before necrosis, all with one exception were homozygous for the *Z. nicaraguensis* allele (data not shown). The one exception could be explained by potential recombination at the necrotic locus.

Mapping QTL for aerenchyma formation

Composite interval mapping detected four QTLs for aerenchyma formation: two on chromosome 1 (tentatively named *Qaer1.02-3*, bin 1.02-3 and *Qaer1.07*, bin 1.07), one on chromosome 5 (*Qaer5.09*, bin 5.09) and one on chromosome 8 (*Qaer8.06-7*, bin 8.06-7) (Fig. 4). These QTLs explain 46.5% of the total phenotypic variance (Table 2). Alleles of *Z. nicaraguensis* increased the level of aerenchyma formation in *Qaer1.02-3* and *Qaer1.07*, while alleles of B64

increased the level of aerenchyma formation in *Qaer5.09*. For *Qaer8.06-7*, a heterozygous genotype decreased the level of aerenchyma formation. The *Qare5.09* could explain the phenotypic transgression seen in Fig. 3 since the *Qaer5.09* genotypes of all four F$_2$ plants with a score exceeding 1.5 are homozygous in B64. The dominance/additive ratio (d/a) can be used as an estimator regarding the mode of gene action with $-0.2 < d/a < 0.2$ indicating additive gene action, $0.2 < |d/a| < 0.8$ suggesting partial dominance, $0.8 < |d/a| < 1.2$ indicating dominant gene action or $|d/a| > 1.2$ suggesting overdominant gene action (Stuber et al. 1987). The gene action of *Qaer1.02-3* and *Qaer5.09* were indicated to be partial dominant, whereas that for *Qaer1.07* and *Qaer8.06-7* suggested overdominance.

The MIM analysis provides evidence for two QTL that were not detected in the CIM analysis. Their positions were on chromosome 1 (*Qaer1.01*) and chromosome 5 (*Qaer5.01*). The r^2 (broad sense heritability) value in the MIM model fitted to the six QTL and their epistatic interaction was 0.621 (Table 2). Epistatic interaction was found between two QTL pairs, *Qaer1.02-3* versus *Qaer8.06-7* and *Qaer5.09* versus *Qaer8.06-7*. The six QTL contributed 56.0% of the total genetic variance and epistatic interaction was estimated at 6.1%.

Differences between CIM and MIM in the LOD score for the putative QTL were found in this analysis (Table 2). This difference is explained by the point that the test under CIM is conditional on all markers while the test under MIM is conditional on all QTL in the model (Zeng et al. 2000).

Discussion

Previous studies have not mapped genes controlling aerenchyma formation in plants. This study has successfully identified and mapped QTLs for aerenchyma formation in roots in non-flooding drained soil conditions. The experiment was performed in a greenhouse and only those individuals that were at a similar growth level were evaluated since the degree of root aerenchyma in non-flooding drained soil conditions can be altered due to various patterns of seedling growth (Mano et al. 2006b). Also, since younger roots tend not to form aerenchyma when in drained soil, even in the best aerenchyma-forming lines
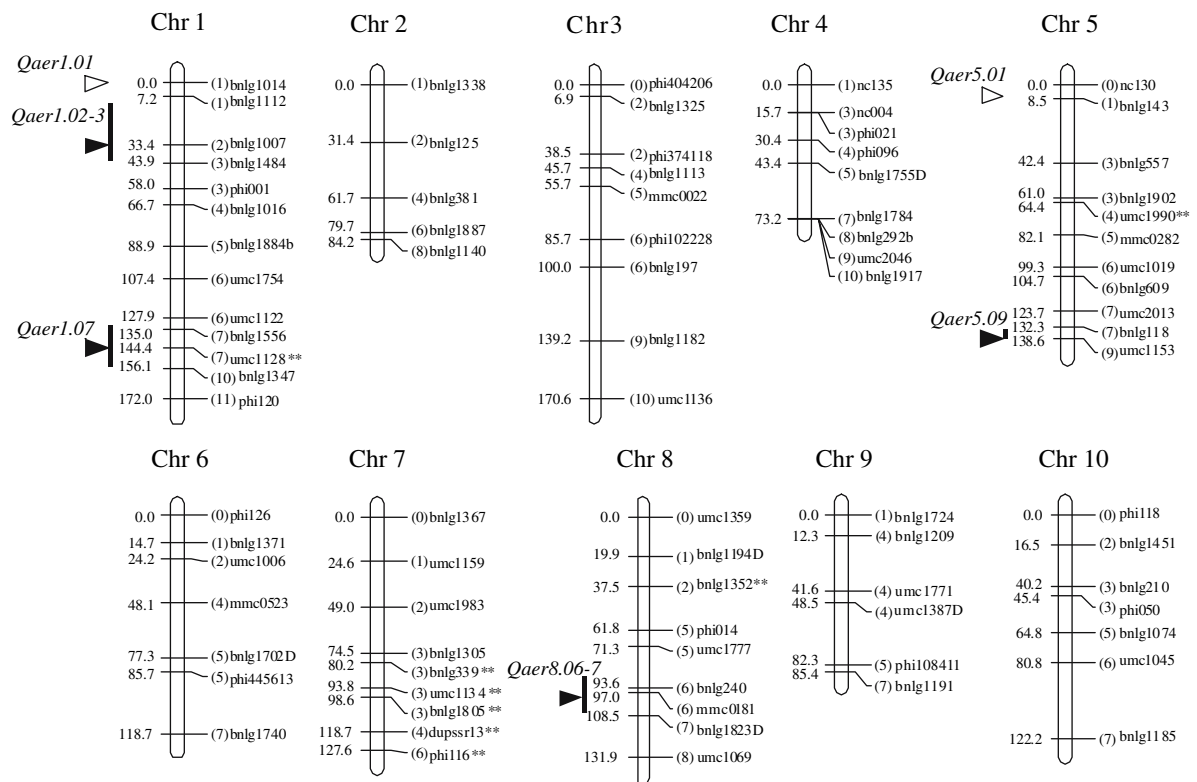
Fig. 4 Chromosome locations of the QTLs for aerenchyma formation in non-flooding drained soil conditions in the B64 × *Z. nicaraguensis* F_2 population identified using composite interval mapping (*CIM*) and multiple interval mapping (*MIM*). Short arms of the chromosome are on the top. The scale represents centimorgan (Haldane units). *Bars* to the *left* of the chromosomes indicate a safe support level of 2-LOD likelihood intervals, and *closed arrowheads* indicate the position of the peak LOD in CIM. *Opened arrowheads* indicate the position of the peak LOD in MIM. Bin numbers are in parentheses before marker names. *Asterisks* indicate markers having distorted segregation (*P* < 0.01)

Table 1 Segregation distortion regions (*P* < 0.01) and the frequency of B64 (*BB*), heterozygous (*BN*), and *Z. nicaraguensis* (*NN*) genotypes in the F_2 population of B64 × *Z. nicaraguensis*

Bin	Marker name	Frequency		
		BB	BN	NN
1.07	umc1128	0.340	0.504	0.156
5.04	umc1990	0.138	0.572	0.290
7.03	bnlg339	0.294	0.598	0.108
7.03	umc1134	0.307	0.620	0.073
7.03	bnlg1805	0.326	0.603	0.071
7.04	dupssr13	0.355	0.638	0.007
7.06	phi116	0.319	0.610	0.071
8.02	bnlg1352	0.187	0.642	0.171

(Y. Mano, unpublished), we did not use the F_2 plants with younger roots even if the individuals were at the same leaf stage. We also used a 30 cm-tall pot to avoid scoring errors; if a shallow pot had been used, roots could have come in contact with each other at the bottom of pot and in such conditions, aerenchyma formation can be induced even in non-aerenchyma forming lines (Y. Mano, unpublished). Our approach enabled 46.5% of the phenotypic variation to be accounted for by four QTLs. Evaluations regarding the expression of these QTLs for aerenchyma formation at the adult plant stage, in flooded and non-flooded conditions will be implemented to confirm the effect of the marker-assisted introgressed QTLs on flooding tolerance. Once introgression is complete, the materials will be subjected to field performance evaluations.

In *Zea*, Ray et al. (1999) suggested that dominant gene(s), that were not mapped, controlled the capacity to form aerenchyma at the adult plant stage in aerated conditions using an F_1 hybrid of *Z. luxurians* × *Z. mays* ssp *mays*. In our study, we detected QTLs for

Table 2 Chromosome locations and mode of gene action of QTLs for root aerenchyma formation in non-flooding drained soil conditions estimated by composite interval mapping (*CIM*) and multiple interval mapping (*MIM*) in the F$_2$ population of the cross between B64 × *Z. nicaraguensis*

QTL	Chr	Position(support interval[b])[a]	Marker interval	LOD	a[c]	d[d]	|d/a|	Mode[e]	Dir[f]	r^{2g}
CIM										
Qaer1.02-3	1	35 (11–43)	bnlg1007–bnlg1484	4.1	0.195	0.065	0.334	pd	N	0.108
Qaer1.07	1	144 (130–154)	umc1128–bnlg1347	5.3	0.134	0.172	1.285	od	N	0.117
Qaer5.09	5	138 (133–138)	bnlg118–umc1153	4.6	−0.205	−0.059	0.287	pd	B	0.109
Qaer8.06-7	8	101 (87–108)	mmc0181–bnlg1823	4.5	−0.114	−0.229	2.014	od	–	0.116
									Total	0.465
MIM										
Qaer1.01	1	0	bnlg1014–bnlg1112	1.7	−0.029	0.188	6.442	od	–	0.071
Qaer1.02-3	1	36	bnlg1007–bnlg1484	2.4	0.163	−0.036	0.219	pd	N	0.104
Qaer1.07	1	144	umc1128–bnlg1347	3.3	0.105	0.182	1.727	od	N	0.145
Qaer5.01	5	6	nc130–bnlg143	1.7	0.083	0.133	1.597	od	–	0.033
Qaer5.09	5	138	bnlg118–umc1153	3.6	−0.191	−0.009	0.047	a	B	0.099
Qaer8.06-7	8	97	mmc0181–bnlg1823	4.9	−0.106	−0.198	1.860	od	–	0.107
Interaction between Qaer1.02-3 and Qaer8.06-7				0.7	−0.185					0.029
Interaction between Qaer5.09 and Qaer8.06-7				1.0	0.206					0.032
									Total	0.621

[a] QTL position in cM from the top of the chromosome

[b] 2-LOD support interval

[c] Additive effect

[d] Dominance effect

[e] 'a' additive gene action, 'pd' partial dominant gene action, 'od' overdominant gene action

[f] Parent contributing higher-value allele, where B = B64, N = *Z. nicaraguensis*

[g] Proportion of phenotyphic variance explained

aerenchyma formation with partial dominance or overdominance effects (Table 2), and the mean value of the capacity to form aerenchyma in the F$_1$ plants resided between B64 and *Z. nicaraguensis* (Fig. 3). Ray et al. (1999) evaluated adult plants, whereas in this study, we evaluated seedlings. The disagreement between the two reports may be explained by the difference in the growth stages at which root aerenchyma was evaluated. The level of aerenchyma formation in roots is minimal at the early seedling stage and aerenchyma are developed gradually (Mano et al. 2006b; Y. Mano, unpublished).

Using *Zea mays* ssp. *mays* × *Tripsacum dactyloides* backcross populations, Ray et al. (1999) suggested that a single major gene controlling aerenchyma formation in non-flooding conditions could be located on the short arm of *Tripsacum* chromosome 16 (Tr16S). Comparative genome analysis between *Zea* and *T. dactyloides* have been reported for some chromosomes using morphological and molecular markers (Eubanks 1997); however, a useful co-linearity analysis of aerenchyma-forming genes between *Zea* and *T. dactyloides* could not be inferred due to lack of information on synteny between *Tripsacum* chromosome 16 and various *Zea* chromosomes.

In maize, some of the biochemical processes in aerenchyma formation (signal transduction; e.g., Jackson and Armstrong 1999) and anaerobic responses (anaerobic proteins; e.g., Subbaiah and Sachs 2003) have been studied. However, useful QTLs (genes) controlling flooding tolerance for practical and applied breeding have not yet been identified. Rice is a cereal grain that exhibits exceptional tolerance to flooding and as a consequence, several QTL associated studies for submergence tolerance have been performed (Siangliw et al. 2003; Xu et al. 2000). In addition, detailed studies have investigated

various cellular events that occur prior to cell collapse (Kawai et al. 1998); however, genetic analysis, gene isolation or expression studies, with regard to the aerenchyma forming process, in rice, has not been forthcoming due to the absence of non-aerenchyma forming lines or mutants. In *Arabidopsis*, similar studies have not been initiated since it does not form aerenchyma and useful mutants are also lacking (Evans 2004). Using the QTL information of aerenchyma formation found in this study, it will be possible to develop near-isogenic lines for the presence-absence aerenchyma, which is useful for molecular and cellular analyses for the trait. For these reasons, the study of aerenchyma development in genus *Zea* is of practical importance.

Of two types of aerenchyma, lysigenous, and schizogenous, summarized by Evans (2004), the aerenchyma observed in our study was classified as lysigenous, based on morphology. Lysigenous aerenchyma formation is promoted by accumulation of endogenous ethylene (e.g., Drew et al. 1979; Justin and Armstrong 1991). Although many ethylene, hypoxia or anoxia-induced genes have been reported for maize, only a few have been located to chromosomes (Sachs et al. 1996). Of these, only one gene, possibly associated with aerenchyma development that has been reported, is a hypoxia-induced gene *xet1*. The *xet1* gene encodes a xyloglucan endotransglycosylase 1 (XET1) and, like aerenchyma, a XET1 transcript was induced by ethylene. The *xet1* has been mapped to maize chromosome 5 (bin 5.03) (Subbaiah and Sachs 2003). This position does not correspond to any of the six QTLs for aerenchyma formation identified in this study. Recently, laser-capture microdissection (LCM) has been applied to plant cells (Nakazono et al. 2003). By comparing gene expression in cortical cells (utilizing LCM and microarray analysis) between B64 and its near-isogenic lines for aerenchyma formation, that we have been developing, it may be possible to isolate aerenchyma-forming genes.

Wild species or ancestral species have provided good sources of genes for improving biotic or abiotic stress tolerances in traditional breeding programs (Harlan 1976; Hoisington et al. 1999). The particular accession of *Z. nicaraguensis* used in this study is adapted to the northwest coastal plain of Nicaragua and can tolerate frequent flooding during a 6-month rainy season (Bird 2000; Iltis and Benz 2000). Under experimental soil flooding conditions, *Z. nicaraguensis* exhibited a higher degree of aerenchyma formation compared to flooding sensitive maize inbred line B64 (Mano et al. 2006b). In addition, this accession has exhibited vigorous growth together with forming a large number of adventitious roots at the soil surface during flooding (Bird 2000) and tolerance to soil flooding at soil reducing or low redox potential (Eh) conditions (Y. Mano, unpublished); and at these conditions, phytotoxins (e.g., Fe^{2+}, H_2S) were induced.

The present study identifies regions on the *Z. nicaraguensis* chromosomes that are involved in the control of aerenchyma formation in roots in non-flooding drained soil conditions. The SSR markers linked to QTLs controlling aerenchyma formation may be a valuable tool for marker-assisted selection without the need to remove plants from the nursery to evaluate the development of aerenchyma channels. QTLs controlling other soil flooding tolerance related trait of adventitious root formation at the soil surface have been previously located on chromosomes 4 and 8 in teosinte *Z. mays* ssp. *huehuetenangensis* (Mano et al. 2005a), and on chromosomes 3, 7, and 8 in tropical maize inbred line Na4 (Mano et al. 2005c). The positions of the QTL for adventitious rooting did not overlap with the *Z. nicaraguensis*' QTL for aerenchyma formation on chromosome 1 (*Qaer1.02-3* and *Qaer1.07*), suggesting that it could be possible to combine two flooding-related traits without concomitant exclusion of the second flooding-tolerant related trait. From this study, it should be possible to transfer QTLs controlling aerenchyma formation in drained soil conditions from *Z. nicaraguensis* to the F_1, F_2 and various backcross generations by marker-assisted selection. The development of near-isogenic lines of B64 is now in progress and they are currently at the BC_3F_1 generation. The pyramiding of the flooding-related QTLs of the capacity to form aerenchyma in drained soil conditions and the ability to form adventitious roots at the soil surface during flooding, and the evaluation of flooding tolerance in developed lines at the field condition, will soon be investigated. We believe this approach will contribute to the practical and applied breeding for flooding tolerance in maize.

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